Reference: *Biol Bull* **177:** 210–217. (October, 1989)

The Egg-Laying Hormone Family: Precursors, Products, and Functions

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Abstract. The marine mollusc *Aplysia* produces an egg-laying hormone (ELH), which induces ovulation and acts on central neurons to effect egg-laying behavior. ELH is synthesized in the neuroendocrine bag cells; it is encoded by the ELH gene, one of a small family of genes, each of which is expressed in a tissue-specific manner. We review what is known about post-translational processing of the ELH precursor, and report the isolation and chemical characterization of ϵ-bag-cell peptide, the seventh peptide product of the ELH precursor to be identified to date. Amino acid compositional and sequence analyses demonstrated that the primary structure of the 19-residue peptide is: NH_2-Ser-Val-Leu-Thr-Pro-Ser-Leu-Ser-Ser-Leu-Gly-Glu-Ser-Leu-Glu-Ser-Gly-Ile-Ser-COOH. Several other ELH-related genes are expressed in the atrial gland, an exocrine organ secreting into the oviduct of *Aplysia*. We review post-translational processing of these ELH-related precursors, and compare the events to those in the neuroendocrine bag cells. Finally, we compare the sequences of six ELH-related peptides from *Aplysia* with one ELH-related peptide (caudodorsal cell hormone) from *Lymnaea* to gain insight into the structure-activity relations of ELH at the ovotestis receptor.

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Abbreviations: A-NTP, postsignal sequence NH_2-terminal peptide encoded by the A-related gene; BCP, bag-cell peptide; B-NTP, postsignal sequence NH_2-terminal peptide encoded by the B-related gene; CDCH, caudodorsal cell hormone; ELH, egg-laying hormone; AP, bag-cell acidic peptide; A-AP, an acidic peptide encoded by the A gene; A-ELH, an ELH-related peptide encoded by the A gene; HPLC, high-performance liquid chromatography; HRBP, histidine-rich basic peptide; PTH, phenylthiohydantoin; TFA, trifluoroacetic acid.

Introduction

The control of egg-laying behavior in the marine mollusc *Aplysia* has been particularly amenable to a multidisciplinary approach aimed at clarifying the cellular and molecular bases of neuroendocrine function. The neurosecretory bag cells are part of the final common pathway leading to egg deposition in these animals. Egg laying is initiated when the bag cells, which are located in the abdominal ganglion (Coggeshall, 1967; Frazier *et al.*, 1967) and are normally electrically silent, begin to fire in a prolonged and synchronous "afterdischarge" that may last 30 min or longer (Kupfermann and Kandel, 1970; Dudek and Blankenship, 1977a, b; Pinsker and Dudek, 1977). Several peptides, including the egg-laying hormone (ELH) (Table I), are released during this activity (Stuart *et al.*, 1980); they may act as classical neurohormones on peripheral targets, such as the ovotestis, or as non-synaptic neurotransmitters within the abdominal ganglion.

The peptides released by the bag cells are encoded by a single gene, known as the ELH gene, which directs the synthesis of a 37-kDa polyprotein precursor (Scheller *et al.*, 1983). A schematic diagram of the precursor (preproELH), as modified by recent peptide studies, is presented in Figure 1. Processing signals in the predicted amino acid sequence of preproELH suggest that it is post-translationally processed to generate nine or more peptide products in addition to the signal sequence. But is this processing scheme actually followed?

Seven of the nine peptides have now been identified in bag-cell extracts or releasates (Table II), and each of them, with the exception of alpha- and delta-bag-cell peptides (α-BCP, δ-BCP), has corresponded to a predicted product of the precursor. The sequence of δ-BCP

is unusual in two respects. First, it contains the only dibasic sequence in the precursor that does not appear to be cleaved during post-translational processing. Second, it is liberated from the processing intermediate (and from α-BCP, which occurs next to it on the precursor) by hydrolysis at a single arginyl residue, the only monobasic site to be cleaved during processing. Of course, this cleavage *might* have been predicted, since the region surrounding this arginyl residue has sequence characteristics that are often observed in association with cleaved monobasic sites (Benoit *et al.*, 1987). Nonetheless, as a result of these unexpected processing events, δ-BCP is 39 rather than 7 residues in length, and α-BCP is 9 rather than 40 residues in length. These observations emphasize the importance of chemically identifying peptide products—*i.e.*, of validating the processing steps that are merely predicted from precursor sequences which are, in turn, predicted from nucleotide sequence analyses of genomic or cDNA clones.

The physiological functions of most of the bag-cell products remain unclear, but their chemical properties (*e.g.*, molecular weight, NH_2- and COOH-terminal modifications) provide important clues as to their stability in biological fluids and thus to the kinds of function(s) that each could serve. Alpha-BCP, for example, probably could not act as a classical neurohormone because of its small size and lack of NH_2- or COOH-terminal modification (Mayeri and Rothman, 1985; Rothman *et al.*, 1987); it is, in fact, rapidly degraded upon release into the extracellular space. On the other hand, α-BCP could, and presumably does, act as a non-synaptic neurotransmitter within the abdominal ganglion (Rothman *et al.*, 1983; Mayeri *et al.*, 1985; Brown and Mayeri, 1986; Sigvardt *et al.*, 1986).

In this report, we describe the chemical characterization of epsilon-bag-cell peptide (ϵ-BCP), the seventh peptide product of the ELH gene to be identified. We also review the chemical characteristics of the ELH peptide family, since this peptide is the most frequently analyzed product of ELH-related genes and it has a well-defined physiological function (or activity)—*i.e.*, the release of mature oocytes from the ovotestis into the ducts of the reproductive tract.

Materials and Methods

Bag-cell clusters and the proximal 1 cm of the pleurovisceral connectives were removed from *A. californica* and immediately stored at −70°C until they were used. Clusters from 50 animals were heated for 3 min at 100°C and homogenized for 1 min (Brinkmann Polytron: setting 4; 4°C) in 15 ml of 1 *M* acetic acid containing 20 m*M* HCl. The extract was centrifuged (48,000 × *g*) for 20 min at 4°C, and the supernatant chromatographed at 4°C on a Sephadex G-50 superfine column (2.5 cm × 50 cm), which had previously been calibrated with molecular weight standards. Fractions were pooled based on absorbance at 280 nm, filtered (0.2 μm pore size), and the filtrate applied to a Supelcosil C18 reversed-phase HPLC column without prior Sep-Pak purification or lyophilization. The column was washed until the absorbance at 220 nm returned to baseline, and was then eluted at a flow rate of 1.0 ml/min with a gradient of Solvent A (0.1% TFA) and Solvent B (acetonitrile containing 0.1% TFA). One-minute fractions (1.0 ml) were pooled based on absorbance, and were subjected to amino acid analysis and automated amino acid sequence analysis. Samples were hydrolyzed with 5.7 *N* HCl *in vacuo* at 107°C for 24 h, and amino acid compositional analyses were carried out on a Beckman 6300 analyzer. The primary structure of the peptide was determined by microsequence analysis using an Applied Biosystems Model 475A Protein/Peptide Sequencer with an on-line 120A microbore PTH analyzer and a Model 900 data processor. The repetitive yield was 86.5%.

Results

An acid extract of heat-treated bag cells was initially chromatographed on a Sephadex G-50 column. The 0-10-kDa peptides in fraction A, a region of low absorbance at 280 nm (not shown), were filtered and fractionated by C18 reversed-phase HPLC using shallow gradient conditions to optimize peptide separation. The eluate was monitored at 220 nm (Fig. 2). Several fractions occurred as relatively broad peaks, primarily due to the shallow gradient conditions employed; however, the relatively low HPLC flow rate and the large amounts of sample loaded onto the column probably also contributed to peak broadening.

Fraction A1, which eluted from 159 through 162 min (Fig. 2), had the following amino acid composition: Thr (0.9), Ser (6.6), Glu (2.0), Pro (0.7), Gly (1.8), Val (0.8), Ile (1.0), Leu (3.8). A comparison of this composition with the reported nucleotide sequence analysis of the ELH gene (Scheller *et al.*, 1983) suggested that fraction A1 was a 19-residue peptide from the ELH precursor. Fraction A1 was subjected to quantitative microsequence analysis (10.0 nmol; 20 cycles), and the resulting amino acid sequence (Table III) was identical to that predicted for residues 156 through 174 of proELH (Scheller *et al.*, 1983). Following convention, this 19-residue peptide was termed ϵ-BCP. Assuming that the COOH terminus of ϵ-BCP was not amidated (since the last residue was not followed by Gly in the precursor), the calculated M_r is 1863. Approximately 2.6 μg (1.39

Table I

Comparison of the primary structures of bag cell, atrial gland and caudodorsal cell peptides[a]

ELH-related peptides	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Aplysia californica ELH[b]	Ile	Ser	Ile	Asn	Gln	Asp	Leu	Lys	Ala	Ile	Thr	Asp	Met	Leu	Leu	Thr	Glu
Aplysia brasiliana ELH[c]	Ile	Ser	Ile	Asn	Gln	Asp	Leu	Lys	Ala	Ile	Thr	Asp	Met	Leu	Leu	Thr	Glu
Aplysia californica [Gln[23], Ala[27]]A-ELH[d]	Ile	Ser	Ile	Asn	Gln	Asp	Leu	Lys	Ala	Ile	Thr	Asp	Met	Leu	Leu	Thr	Glu
Aplysia californica [Ala[27]]A-ELH[d]	Ile	Ser	Ile	Asn	Gln	Asp	Leu	Lys	Ala	Ile	Thr	Asp	Met	Leu	Leu	Thr	Glu
Aplysia californica A-ELH[d]	Ile	Ser	Ile	Asn	Gln	Asp	Leu	Lys	Ala	Ile	Thr	Asp	Met	Leu	Leu	Thr	Glu
Aplysia parvula ELH[e]	Ile	Ser	Ile	Asn	Gln	Asp	Leu	Lys	Ala	Ile	Ala	Asp	Met	Leu	Ile	Val	Glu
Lymnaea stagnalis CDCH[f]	Leu	Ser	Ile	Thr	Asn	Asp	Leu	Arg	Ala	Ile	Ala	Asp	Ser	Tyr	Leu	Tyr	Asp

[a] Boxed residues indicate positions where the peptides differ from *A. californica* ELH. All of the ELH-related peptides are presumed to be amidated based on molecular genetic (Mahon *et al.*, 1985; Nambu and Scheller, 1986; Scheller *et al.*, 1983; Shyamala *et al.*, 1986) and peptide studies (Chiu *et al.*, 1979; Ebberink *et al.*, 1985).

[b] Determined by Chiu *et al.* (1979).

[c] Determined by Nagle *et al.* (1988b).

[d] Determined by Nagle *et al.* (1986) and Rothman *et al.* (1986).

[e] Predicted from nucleotide sequence analysis of an *A. parvula* bag cell genomic ELH clone (Nambu and Scheller, 1986).

[f] Determined by Ebberink *et al.* (1985).

nmol) of ϵ-BCP was recovered from each pair of bag-cell clusters. The physiological function of this peptide is not known.

The prominent fraction which eluted from 115 through 124 min (Fig. 2) was also examined. Amino acid compositional and sequence analyses demonstrated that this fraction corresponded to the myoactive 43-residue histidine-rich basic peptide (HRBP) of neurons R3-R14 (Nagle *et al.*, 1989). A wealth of data, both anatomical (Frazier *et al.*, 1967) and biochemical (Newcomb and Scheller, 1987), indicates that the R3-R14 neurons have axon terminals in the neurohemal region surrounding the bag cells.

Discussion

Chemical characterization of the peptide products predicted from the ELH gene expressed in the bag cells provides definitive information about post-translational processing in these model neuroendocrine cells. Of the seven peptides characterized to date, five are processed as predicted, in response to signals that have been empirically determined from similar studies in other systems. However, two peptides comprising contiguous segments of preproELH, are not processed as initially predicted: a dibasic site in the middle of the δ-BCP sequence is not cleaved, while a monobasic Arg separating δ-BCP from α-BCP is. Examination of the characteristics of these sites in greater detail, and their comparison to qualitatively similar sites in the precursor that are processed differently, may provide insights that will allow increasingly accurate predictions of post-translational processing events to be made in the future. Moreover, since the bag-cell ELH gene is only one of a small family of structurally related genes that are expressed in a tissue-specific manner in *Aplysia* (Scheller *et al.*, 1983), peptide characterization studies may help to distinguish between general and tissue-specific processing events. Comparisons of specific peptide sequences (*e.g.*, the ELH-related peptides) from different tissues and species may be equally useful for preliminary structure-activity analyses of peptide action.

The atrial gland, an exocrine organ secreting into the oviduct of *Aplysia* (Arch *et al.*, 1980; Beard *et al.*, 1982; Painter *et al.*, 1985), expresses several ELH-family genes (Scheller *et al.*, 1983; Mahon *et al.*, 1985). The peptide products of this gland pharmacologically elicit egg deposition when injected into a receptive animal (Arch *et al.*, 1978), but have no known physiological function inside the organism. Recent experiments suggest that the secreted peptides may be deposited onto the egg cordon as it is transported through the oviduct, and may mediate the sexual and social behaviors often associated with egg laying and egg cordons (Painter *et al.*, 1989).

Table I (*Continued*)

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Gln	Ile	Arg	Glu	Arg	Gln	Arg	Tyr	Leu	Ala	Asp	Leu	Arg	Gln	Arg	Leu	Leu	Glu	Lys-NH_2
Gln	Ile	Arg	Glu	Arg	Gln	Arg	Tyr	Leu	Ala	Asp	Leu	Arg	Gln	Arg	Leu	Leu	Glu	Lys-NH_2
Gln	Ile	Gln	Ala	Arg	Gln	Arg	Cys	Leu	Ala	Ala	Leu	Arg	Gln	Arg	Leu	Leu	Asp	Leu-NH_2
Gln	Ile	Gln	Ala	Arg	Arg	Arg	Cys	Leu	Ala	Ala	Leu	Arg	Gln	Arg	Leu	Leu	Asp	Leu-NH_2
Gln	Ile	Gln	Ala	Arg	Arg	Arg	Cys	Leu	Asp	Ala	Leu	Arg	Gln	Arg	Leu	Leu	Asp	Leu-NH_2
Gln	Lys	Gln	Glu	Arg	Glu	Lys	Tyr	Leu	Ala	Asp	Leu	Arg	Gln	Arg	Leu	Leu	Asn	Lys-NH_2
Gln	His	Trp	Leu	Arg	Glu	Arg	Gln	Glu	Glu	Asn	Leu	Arg	Arg	Arg	Phe	Leu	Glu	Leu-NH_2

Each of the ELH-family genes expressed in the atrial gland encodes a large polyprotein precursor. Two of the precursors, preproA and preproB, are diagrammed in Figure 1; the diagrams are based on nucleotide sequence analyses of genomic and cDNA clones (Scheller *et al.*, 1983; Mahon *et al.*, 1985) and have been modified by peptide sequence analyses (Heller *et al.*, 1980; Nagle *et al.*, 1986, 1988c; Rothman *et al.*, 1986). The products that have been isolated and chemically characterized are summarized in Table IV.

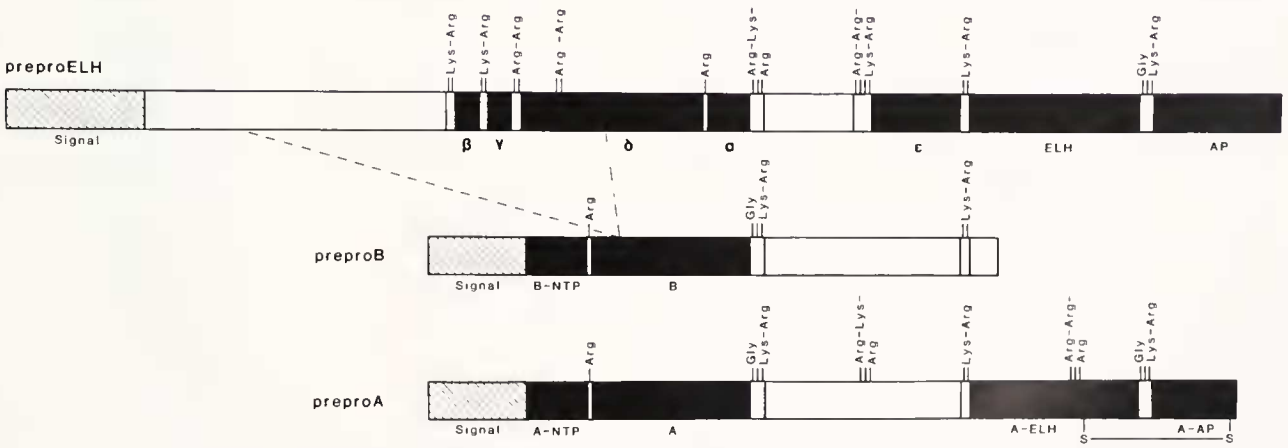

Figure 1. Schematic diagram of *Aplysia californica* preproELH, preproB, and preproA as predicted from nucleotide sequence analyses of genomic and cDNA clones (Scheller *et al.*, 1983; Mahon *et al.*, 1985) and modified by peptide studies (Nagle *et al.*, 1986, 1988c; Rothman *et al.*, 1986). Signal peptides are represented by hatched boxes. Peptides that have been identified in extracts or releasates are indicated by black boxes and have been labeled. Known or predicted mono-, di-, tri-, and tetrabasic cleavage sites, as well as the Gly-Lys-Arg signal for COOH-terminal amidation, are shown. Abbreviations are defined in a footnote on the first page of this paper.

Table II

Peptides derived from Aplysia californica *bag-cell preproELH*[a]

Peptide	Source	Means of identification	Number of residues	Function or pharmacological action
α-BCP[b]	Extracts	Sequence	7, 8, or 9	Inhibits LUQ cells Inhibits/excites bag cells
β-BCP[c]	Releasates	Comigration with Standard	5	Excites L1, R1, and bag cells in abdominal ganglion
λ-BCP[c]	Releasates	Comigration with Standard	5	Excites bag cells
δ-BCP[d]	Extracts	Sequence	39	Stimulates Ca flux into mitochondria of albumen gland secretory cells
ε-BCP[d]	Extracts	Sequence	19	Not known
ELH[e]	Extracts	Sequence	36	Induces egg release from gonad, excites R15 and LLQ cells in abdominal ganglion and B16 in buccal ganglion
AP[f]	Extracts	Sequence	27	Not known

[a] A schematic diagram of preproELH is presented in Figure 1. Abbreviations are defined in a footnote on the first page of this paper.
[b] Rothman *et al.*, 1983.
[c] Rothman *et al.*, 1985.
[d] Nagle *et al.*, 1988a.
[e] Chiu *et al.*, 1979.
[f] Scheller *et al.*, 1983.

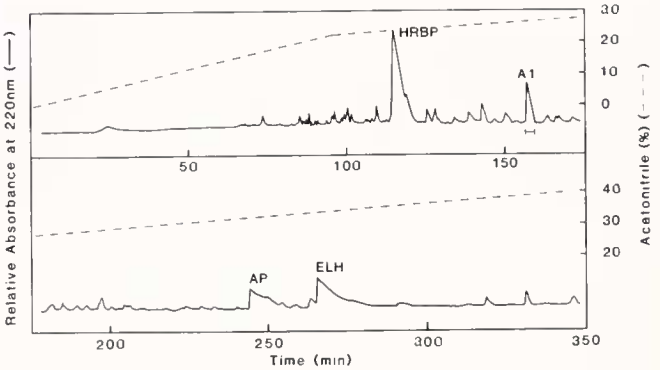

Figure 2. Reversed-phase HPLC purification of 0-10-kDa peptides from *Aplysia californica* bag cells. An extract of the bag cells was initially fractionated by Sephadex G-50 column chromatography to generate fraction A, which contained the 0-10-kDa peptides (not shown). Fraction A was filtered and then subfractionated by C18 reversed-phase HPLC using two linear gradients of 0.1% TFA and acetonitrile containing 0.1% TFA (0–22% in 96 min, 22–44% in 310 min) to generate fraction A1. A single elution profile has been divided into two panels. Abbreviations are defined in a footnote on the first page of this paper.

The atrial gland ELH-related peptides are 36-residue peptides that are identical to bag-cell ELH at residues 1–19 and at six of eight COOH-terminal residues (Table I); they are approximately equipotent to bag-cell ELH in eliciting egg deposition. Nevertheless, the atrial gland peptides differ from their homolog in the bag cells in several important characteristics. First, there is a potential tribasic cleavage site in A-ELH and [Ala^{27}]A-ELH which is missing from bag-cell ELH (Fig. 1; Table I). Second, each of the three atrial gland peptides is disulfide-bonded to an 18-residue acidic peptide, A-AP, that is located adjacent to it in the precursor. This linkage, through Cys^{25} of the ELH-related molecules, may sterically inhibit cleavage at the tribasic sequence (Arg^{22}-Arg^{23}-Arg^{24}) and explain why the site is not used during post-translational processing. The function of this heterodimeric complex is not known, but it is approximately equipotent to bag-cell ELH in eliciting egg deposition. Finally, a proportion of the A-AP/A-ELH and A-AP/[Ala^{27}]A-ELH complexes are further processed in the atrial gland by a renin-like enzyme, with cleavage occurring at the Leu^{14}-Leu^{15} and Leu^{33}-Leu^{34} bonds of the ELH-related sequences. The Leu^{11}-Leu^{12} bond in A-AP is not cleaved, however, perhaps due to differences in secondary structure that have been predicted to occur in these regions by Chou-Fasman analysis (α-helix in the ELH-related peptides, but β-sheet in A-AP) (Nagle *et al.*, 1986). Cleavage of the

Table III

Automated sequence analysis of fraction A1[a]

Edman cycle	Residue (pmol) A1
0[b]	— (10000)
1	Ser (2819)
2	Val (3836)
3	Leu (5036)
4	Thr (3540)
5	Pro (4459)
6	Ser (2317)
7	Leu (3424)
8	Ser (1890)
9	Ser (1433)
10	Leu (1960)
11	Gly (1383)
12	Glu (1148)
13	Ser (632)
14	Leu (1029)
15	Glu (643)
16	Ser (342)
17	Gly (434)
18	Ile (358)
19	Ser (127)

[a] Results obtained with gas-phase microsequencer. Phenylthiohydantoin (PTH) amino acids were quantitated by HPLC.
[b] Initial amount of peptide (pmol) applied to sequencer.

Table IV

Peptides derived from the ELH-family genes expressed in the Aplysia californica *atrial gland*[a]

Peptide	Method of identification	Number of residues	Pharmacological action
A-NTP[b]	Sequence	13	Not known
B-NTP[b]	Sequence	13	
A[c]	Sequence	34	Bag-cell activation
B[c]	Sequence	34	
A-ELH[d,e]	Sequence	36	Egg release from ovotestis
[Ala^{27}]A-ELH[d,e]	Sequence	36	
[Gln^{23}, Ala^{27}]A-ELH[d,e]	Sequence	36	
A-ELH-(1–14)[f]	Composition	14	Not known
A-ELH-(15–36)[b]	Sequence	22	
A-ELH-(1–33)[b]	Composition	33	
[Ala^{27}]A-ELH-(15–36)[b]	Sequence	22	
[Ala^{27}]A-ELH-(1–33)[b]	Composition	33	
[Gln^{23}, Ala^{27}]A-ELH-(16–36)[b]	Sequence	21	
A-AP[d,e]	Sequence	18	Not known

[a] A schematic diagram of two of the polyprotein precursors, preproA and preproB, is presented in Figure 1. Abbreviations are defined in a footnote on the first page of this paper. Peptides were isolated from tissue extracts.
[b] Nagle *et al.*, 1988c.
[c] Heller *et al.*, 1980.
[d] Nagle *et al.*, 1986.
[e] Rothman *et al.*, 1986.
[f] Rothman *et al.*, 1984.

Leu^{14}-Leu^{15} bond of the ELH-related peptides abolishes egg-laying activity. Since these atrial gland peptides do not induce egg deposition *in vivo*, however, it is not clear whether this processing step represents an activation or inactivation with regard to their actual function. It is important to note that the Leu^{14}-Leu^{15} and Leu^{33}-Leu^{34} bonds of bag-cell ELH are not cleaved during processing, even though each is predicted to occur in an α-helical segment of the molecule. The renin-related proteolysis thus appears to be a tissue-specific processing event, and one that would not be predicted to occur based on nucleotide sequence analyses of genomic or cDNA clones.

In spite of the differences in their sequences, ELH and the atrial gland ELH-related peptides are predicted to have the same secondary structure: two regions of strong α-helical potential (residues 6–21 and 26–36) separated by a β-bend (residues 22–25) (Nagle *et al.*, 1986). These observations suggest that ELH may be a U-shaped peptide, and that egg-laying activity in *Aplysia* may be correlated with conservation of the NH_2- and COOH-terminal regions of the molecule. Two additional ELH-related sequences have been reported to date—the *A. brasiliana* bag-cell ELH, determined by direct chemical characterization of the isolated peptide (Nagle *et al.*, 1988b), and the *A. parvula* bag-cell ELH, predicted from nucleotide sequence analyses of genomic clones (Nambu and Scheller, 1986). Both are 36 amino acids in length (Table I). The sequence of *A. brasiliana* ELH is identical to that of *A. californica* bag-cell ELH and so does not provide any further information about regions of the peptide important for receptor recognition and activation. The *A. parvula* ELH, in contrast, is only 78% identical to *A. californica* bag-cell ELH, and it displays the same pattern of residue conservation observed with the *A. californica* peptides: it is identical to all five sequenced peptides at 13 of 14 NH_2-terminal positions and at 6 of 8 COOH-terminal positions, but differs significantly from them in the intervening region (Table I).

More detailed structure-activity information has been obtained recently using synthetic ELH and analogs to induce egg deposition (Strumwasser *et al.*, 1987). These studies confirm that peptide chain-length is important for egg-laying activity. Removal of the NH_2-terminal Ile, or extension of the COOH terminus by one residue (ELH-Gly^{37}), results in a loss of egg-laying activity. In contrast, ELH-(1–34) and ELH-(1–35) are at least moderately active, indicating that the COOH-terminal amide is not essential for biological activity, and that the identities of the amino acids at positions 35 and 36 may be relatively unimportant. This conclusion is consistent with the comparative peptide studies outlined above, since substitutions at positions 35 and 36 of the ELH-

related peptides did not significantly decrease biological activity relative to *A. californica* bag-cell ELH (Table I).

Other positional requirements are less clear, however. The amino acids critical for receptor recognition and activation are probably concentrated at positions 1–10 and 29–34, since all six *Aplysia* ELH-related sequences are identical at these positions. If we extend the comparison to include the caudodorsal cell hormone (CDCH) of the freshwater pulmonate *Lymnaea stagnalis* (Table I; Ebberink *et al.*, 1985), an interesting pattern emerges. (CDCH is a 36-residue peptide secreted by the neuroendocrine caudodorsal cells during a burst of activity comparable to a bag-cell afterdischarge; it induces ovulation and is homologous to ELH.) Only 13 amino acid residues are conserved in all seven peptides, and 10 of the 13 (77%) occur in the regions encompassing residues 1–10 and 29–34. The number increases to 11 of 13 if we include positions 11 and 12 in the comparison, and four of them are charged (Asp^{6}, Asp^{12}, Arg^{30}, Arg^{32}). These observations strengthen the notion that the regions encompassing residues 1–12 and 29–34 may be important for receptor recognition and activation, and suggest that these positions should be modified in ELH analogs for future structure-activity studies.

Acknowledgments

This investigation was supported by NSF BNS 85 17575 (GTN) and BBS 87 11368, and by NIH NS 22079 (SDP), NS 23169 (JEB), and NS 11255.

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